Electrophoretic separation of large DNAs using steric confinement

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Abstract

We report an alternative method for electrophoretic separation of large DNAs using steric confinement between solid walls, without gel or obstacles. The change of electrophoretic mobility *vs* confinement thickness is investigated using fluorescence video microscopy. We observe separation at small confinement thicknesses followed by a transition to the bulk behaviour (no separation) at a thickness of about 4 μm (a few radii of gyration for the studied DNA chains). We present tentative explanations of our original observations.

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1 Introduction

Capillary electrophoresis in polymer solutions has become an alternative method of gel electrophoresis for DNA separation (review in [1]). Simultaneously, there has been a stimulating development of new methods using new geometries. For instance, separation of DNA adsorbed on a flat surface [2] exploits the dependence of the adsorption-desorption kinetics on chain length; microlithography has been used to create topological defects and entropic trapping [3]. Is it possible to find a simpler way of separation? Could the sole geometrical confinement be sufficient? Two groups have worked in this direction. Roeraade et $al.$ [4] have separated a mixture of HindIII-digested λ-DNA fragments, using capillaries with diameters of order of two radii of gyration (R_g) of the largest fragment. Iki et $al.$ [5] have used 20 μm-diameter capillaries. Both groups have explained the separation using electrostatics. Roeraade has considered that the smallest molecules are repelled towards the capillary center since walls and DNA are both negatively charged. He has calculated that capillaries with small inner diameter (< 1 μm) filled with low ionic strength solution lead to an electro-osmotic flow (EOF) with a Poiseuille profile: the smallest molecules experience the fastest stream lines and are eluted first. Iki has considered that the smallest molecules have a smaller mobility in the vicinity of the wall because of the screening by the surface counter-ions; with the EOF in the opposite direction of the electrophoretic force, the smaller the molecule, the higher velocity in the capillary.

The present paper reports experiments performed in different conditions (higher ionic strength than [4], tighter confinement and much lower electric fields) that exhibit totally different behaviours.

2 Materials and methods

Confinement is achieved using a home-built cell (Fig. 1). Two glass plates (microscopy slides) are separated by the DNA solution and connected to two reservoirs, hosting Pt electrodes to apply an electric field (using an ISO-TECH power supply). The glass plates are washed with 1 % RBS solution (an alkaline powerful industrial detergent from Roth-Sochiel) in a sonication bath (Branson 1200) for 10 min, and then thoroughly rinsed with ultra pure water (Millipore Milli-Q). The confinement gap is filled with a solution 36 pmol·l^{-1} for λ-DNA (48.5 kbp from Biolabs) or 11 pmol·l^{-1} for T2-DNA (164 kbp from

Sigma) in 10^{-2} mol· l^{-1} TBE buffer solution pH 8.3. Both concentrations correspond to the same mass concentration, 1.2 μg·ml^{-1} (dilute regime). The DNAs' radii of gyration are estimated from the Benoit-Doty relation [6]: $R_g \simeq \sqrt{L_c l_p/3}$ (with L_c the contour length and l_p the persistence length [7]). This gives $R_g = 0.56$ μm for λ-DNA ($L_c = 16.5$ μm and $l_p = 50$ nm) and $R_g = 1.13$ μm for $T2$-DNA ($L_c = 55.8$ μm and $l_p = 50$ nm). The DNA molecules have been labeled with a fluorescent dye (YOYO-1, Molecular Probes): 1 dye per 50 base-pairs. This very small dye content ensures that the characteristics of DNA (charge, persistence length etc.) remains unaltered. The images are taken at 25 frames per second during 8 seconds using a silicon intensified target (SIT) camera (Hamamatsu) fitted to a microscope (Leica). No electric field is applied during the first two seconds to estimate the possible convection in the sample. Five field values (from $\simeq$ 2 to 8 V·cm^{-1}) have been used and the electrophoretic mobility is derived from the slope of the linear variation of velocity with applied field.

The image analysis is performed as follows. In a first set of experiments, using solutions containing only one individual species (either λ-DNA or $T2$-DNA), we have derived the velocities of the chains from the correlation of successive frames. In a second set of experiments we have investigated mixtures of both DNAs: we have determined the position [8] *and* the size of the molecules [9] to derive the 2D histogram of velocity *vs* radius and the mean velocity of each population.

The confinement thickness is determined from the electrical resistance of the confined liquid film. The measurement of the current gives a constant and reproducible value 30 min after tightening the plates, indicating mechanical equilibrium. The calibration is done using spacers of 0.5 and 2 μm (monodisperse polystyrene beads) and also gives the conductivity C of the solution. Indeed the resistance R of the film is related to the thickness e by the relation $R = \dfrac{1}{C}\dfrac{L}{el}$ where L is the length of the plate along the direction of the electric field, l is the width of the plate normal to this field. We obtain a conductivity of 1.9 ± 0.2 mS·cm^{-1} in fairly good agreement with the 2.2 mS·cm^{-1} value measured with a conductimeter (Fisher scientific Accumet AR20) at room temperature. We set the gap thickness by tightening uniformly the two plates together. The accuracy of the thickness measurement is around 10 % and the thickness is uniform within ± 0.1 μm. The excess of solution leaving the gap on the side of the plates during tightening is carefully removed to avoid any shortcut for the electric current. The evaporation of the solvent

is negligible because of the area of solvent exposed to air is very small. Finally, the variations of the thickness explored by DNA molecules during an experiment of 8 s are also considered as negligible.

3   Results

Fig. 2 represents the measured mobility μ as a function of the confinement thickness e (first set of experiments). A first and striking feature is that μ-values are positive: negatively charged DNAs move towards the cathode in the same direction as the electric field $\mathbf{E}$. This behaviour is caused by the presence of an electro-osmotic flow (EOF): glass plates are negatively charged at $pH = 8.3$ and their positively charged counter-ions, dragged by the electric field $\mathbf{E}$, induces a plug flow of the solution with velocity v_{EOF} towards the negative electrode. This can be characterized by an electro-osmotic mobility $\mu_{EOF} > 0$ defined by $v_{EOF} = \mu_{EOF}E$. When this mobility is larger than the intrinsic (and negative) electrophoretic mobility of DNA, the effective mobility is positive.

We distinguish two regimes for $\mu(e)$. In a first regime, for *small* thicknesses ($e < 4$ μm), μ increases with a large dependence on chain length. Although $T2$-DNA is longer, it has a larger mobility than λ-DNA: for instance, for $e = 2$ μm, $T2$-DNAs move more than twice faster than λ-DNAs. EOF reverses the order of elution; this effect has been already observed by Barron [10] during DNA electrophoresis in very dilute polymer solutions using uncoated capillary glass tubes.

In a second regime, for thicknesses larger than 4 μm, μ is constant and equal to $+(1 \pm 0.3)10^{-4}$ cm$^2\cdot$ V$^{-1}\cdot$s^{-1} and neither depends on chain length nor confinement thickness.

The first and second regime are separated by a transition region with an important reduction of the mobility.

In the second set of experiments, we have measured the mobilities in a 50-50 mixture of the original $T2$-DNA and λ-DNA solutions. The weight concentration is thus still 1.2 μg$\cdot$ml^{-1}, but since λ-DNAs are 3 times shorter than $T2$-DNAs, the solution contains an average $T2/\lambda$ chain ratio of 1:3. Fig. 3 represents the 2D histogram of the velocity *vs* measured radius for different confinement thicknesses, showing two well-defined populations, with the expected 1:3 ratio. Note that the two clusters are centered on about 1.3 μm and about 1.6 μm instead of the calculated R_gs (0.56 μm and 1.13 μm). Decon-

volution of the measured radii using the formalism of point spread function leads to (resp.) 0.6 and 1.1 μm, in excellent agreement with the calculated values. The mean velocities of the two populations have been extracted from the centers of mass of each population.

Mobility values are summed up in table 1. In the mixture, the overall behaviour is identical to the one described for the first set of experiments (two regimes for the mobility and T2-DNA faster than λ-DNA). Both λ-DNA and T2-DNA go faster when mixed. This observation is due to the procedure of the determination of the mobilities in the mixture which leads to a larger uncertainty: we need to distinguish two populations and then calculate the mobility of each population rather than measure the mean mobility of the assembly of molecules seen on the images. We also observe that the smaller (λ) DNA chains move significantly faster in mixture than in pure solutions, maybe entrained by the larger (T_2) chains.

Finally, in order to prove that EOF indeed reverses the order of elution, we have coated the glass plates with poly(N,N,-dimethylacrylamide) (PDMA), a *neutral* polymer, in order to eliminate the EOF. We let the polymer adsorb on the glass surfaces by incubating the glass plates overnight in a PDMA solution (1% w/w in water)[10], then rinsed with distilled water. With the PDMA coating, we observe that the DNAs move towards the anode (negative mobilities on fig. 3.E) which means that the EOF as been removed by the coating. As expected, the absolute amplitudes of the two DNA mobilities are reversed: λ-DNA moves faster than T2-DNA with mobilities of $+(3.1 \pm 0.2)10^{-4}$ cm$^2 \cdot$V$^{-1} \cdot$s^{-1} and $+(2.2 \pm 0.2)10^{-4}$ cm$^2 \cdot$ V$^{-1} \cdot$s^{-1} respectively.

4   Discussion

The experiments performed in capillaries and reported by Roeraade *et al.* [4] and by Iki *et al.* [5] also shown a separation but with different features and interpretations as mentioned in the introduction. But let us notice that our experimental conditions are significantly different:

- we have used a confinement between two plates and not in a capillary tube;

- the diameters of the capillaries in [4] and [5] are always larger than the largest chain fragments. Anyway, on decreasing the confinement

(in our second regime), we never observe the behaviours described by Roeraade and Iki;

- they have used high electric fields (170 to 200 V· cm^{-1}) which may lead to Joule heating, resulting in radial convection in the capillary. Such an effect is not present in our experiments (maximum field $\simeq 8$ V· cm^{-1});

- in the case of Roeraade's work, it was observed that a good separation is obtained only at low ionic strength (salt concentration close to 50 μM).

All these differences explain that we observe original behaviours and we have thus to find different migration mechanisms. In the following, we present simple models combining the electric force applied on the DNA and the friction of the molecule on the solvent and on the walls to explain the observed behaviours.

In the second regime, the effective mobility μ remains constant ($= +(1 \pm 0.3)10^{-4}$ cm^2·V^{-1}·s^{-1}) and does not depend on the DNA length: this is characteristic of a bulk regime (no separation) with an overall displacement of the solvent by the EOF. This yields:

$$\mu = \mu_e + \mu_{EOF} \tag{1}$$

where $\mu_e < 0$ is the intrinsic mobility of the DNAs (in bulk solution and without EOF) and is mass independent (free draining conditions). μ_{EOF} is related to the zeta potential ζ_S (< 0) of the glass surface by $\zeta_S = -(\eta\mu_{EOF})/(\epsilon_0\epsilon_r)$ with ϵ_0 the vacuum permittivity, ϵ_r and η the dielectric constant and viscosity of the solution. Using $\mu_e = -4\,10^{-4}$ cm^2· V^{-1}· s^{-1} [11, 12](for a similar ionic strength), we find $\mu_{EOF} = +(5 \pm 0.3)10^{-4}$ cm^2·V^{-1}·s^{-1}, in agreement with the values reported in [10, 11, 12]($+5.6\ 10^{-4}$ cm^2·V^{-1}·s^{-1}) in similar conditions. This allows a determination of the zeta potential of the glass plates: $\zeta_S \simeq -70$ mV (with $\eta = 10^{-3}$ N· m^{-2}·s, $\epsilon_0 = 8.8\,10^{-12}$ C^2· N^{-1}· m^{-2} and $\epsilon_r = 80$), in good agreement with reported values [13].

On increasing the confinement (decreasing the gap e between plates), we observe an important *increase* of the mobility. Assuming that the EOF is constant (since e is much larger than the Debye length $\kappa^{-1} \simeq 2$ nm), this can be achieved solely by a decrease of the electric force acting on DNAs. This may be realized likely through a local screening of the DNA charges within the proximal layers of the glass plates but we have no model to discuss quantitatively this possibility.

In the first regime, the mobility increases with the gap thickness e and decreases with the chain length N, and our set-up separates DNAs of different length. We present a tentative theoretical model to describe the dependence of the effective mobility on the confinement thickness e and the chain length (number of monomers N).

Using the results of [14], the effective mobility of the confined DNA is given by:

$$\mu = \frac{\mu_e + \mu_{EOF}}{1 + \xi_1/\xi} \tag{2}$$

$\xi_1 \sim \eta N_1 a$ is the friction coefficient of the N_1 sliding monomers (of size a) on the cell walls and ξ is the friction coefficient of the remaining $N - N_1$ monomers in the gap between the two walls. In absence of specific adsorption, $N_1 = Na/e$ [15] and then $\xi_1 \sim \eta Na^2/e$; inside the gap, the DNA chain behaves as an impermeable coil [14] and ξ is proportional to $N^{1/2}$ (gaussian ideal chain) or to $N^{3/4}a^{5/4}e^{-1/4}$ (taking into account monomers excluded volume). Eq. 2 then does predict an increase of the mobility with thickness e but fails to describe the N dependence.

Assuming $N_1 \sim N^{1/2}$, as suggested in [2] for unconfined DNA, we can satisfactorily describe the experimental data if we consider that the chain inside the gap behaves as a free draining coil (*ie* $\xi \sim N$) but Eq. 2 then fails to describe the thickness dependence. We have experimentally addressed the possibility of strong DNA adsorption that could give special N_1 dependence on N. Using AFM (atomic force microscopy), we have determined that the maximum roughness of the glass plates is about 2 nm: plate surfaces are smooth at the scale of the inter-plate gap size and DNA separation cannot be attributed to chain trapping on surface defects. Furthermore we have not observed J or U DNA shapes, characteristic of such trapping [16]. Moreover, scanning the samples in the z-direction with an optical microscope does not reveal any particular DNA adsorption on the glass plates.

5   Conclusion

We have investigated the mobility of DNA chains confined between two solid walls. The EOF reverses the order of elution of the DNA chains. We have shown that steric confinement constitutes a simple and *fast* technique for separation of large DNA, without gel or pulsed field, using weak electric fields. The interpretation of the underlying mechanisms is still opened.

Acknowledgments

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Table 1: Mobilities of λ- and T2-DNA for different confinements, measured in a pure solution and in the mixture (*interpolated).

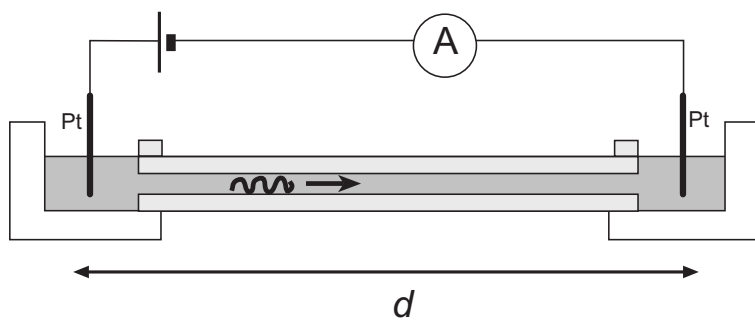| Species	Thickness (μm)	μ (pure) (10^{-4} cm^2·V^{-1}·s^{-1})	μ (mixture) (10^{-4} cm^2·V^{-1}·s^{-1})
λ DNA	1.2	0.6	1.3 ± 0.1
	2.3	1.7*	2.4 ± 0.1
	5	0.8*	1.2 ± 0.1
	8	1.1	1.0 ± 0.1
T2 DNA	1.2	1.7	1.4 ± 0.1
	2.3	3.0*	3.2 ± 0.1
	5	1.1	1.3 ± 0.1
	8	1.1	1.0 ± 0.1

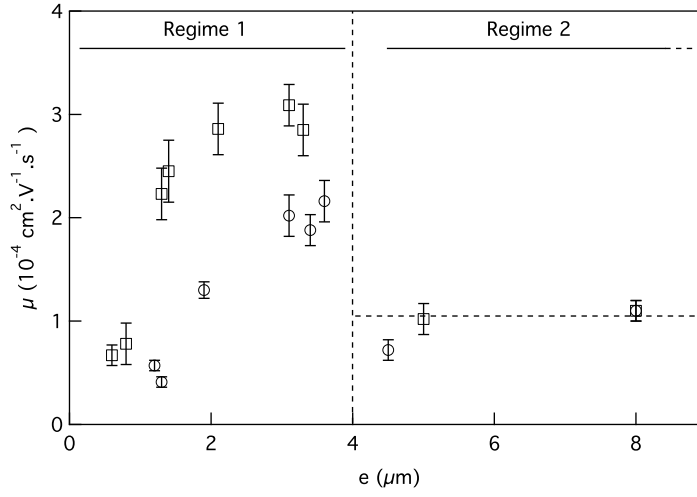Figure 1: Schematic representation of the measurement cell (the distance d between electrodes is 8 cm)

Figure 2: Mobilities μ of λ ($\circ$) and $T2$ ($\square$) DNA *vs* confinement thickness e. The vertical dashed line represents the transition between the two regimes. The horizontal dashed line is a eye guide of the constant mobility in the second regime.
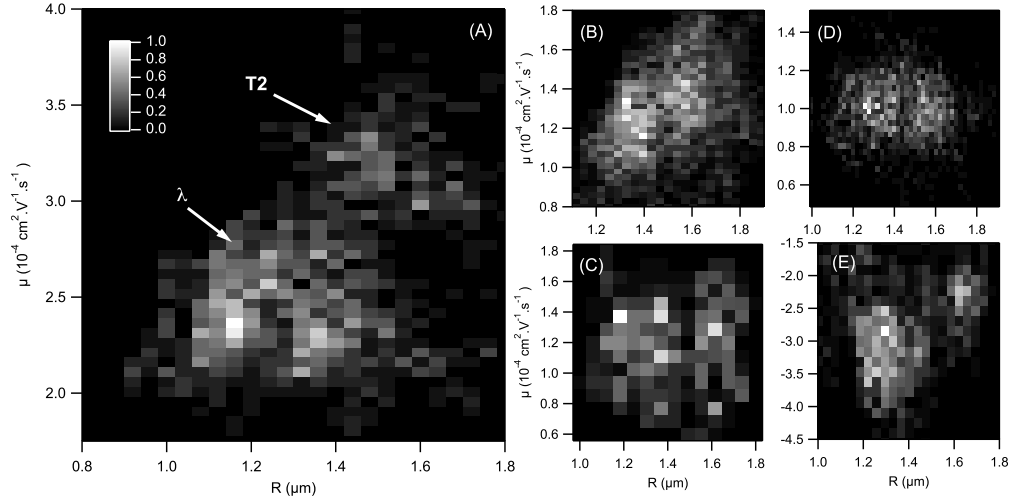
Figure 3: Normalized 2D histogram of the mobility of molecules *vs* their radius. Confinement thicknesses are (μm): (A) 2.3, (B) 1.2, (C) 5, (D) 8 and (E) 3 with PDMA coating. Electric voltage is 60 V. Bin sizes are (units μm and 10^{-4} cm^2·V^{-1}·s^{-1}): (A) 0.04 and 0.05, (B) 0.04 and 0.03, (C) 0.06 and 0.08, (D) 0.02 and 0.03 and (E) 0.03 and 0.08. The mobilities are defined as positive if the molecules move to the positive electrode. Notice the negative sign of the mobility when using PDMA coating which indicates that molecules go in the opposite direction compared to the situation with EOF.